GUIDE TO PIPETTING

Third Edition

GILSON®

HOW THE PIPETTE STORY BEGAN...

Over a century ago, Louis Pasteur invented the glass Pasteur pipette to reduce contamination when transferring samples. The Pasteur pipette is still in use today.

The next notable advancement in pipetting occurred in the late 1950s with the introduction of a handheld, piston-operated pipette as a safe alternative to potentially dangerous mouth pipetting. The first handheld pipettes were fixed volume pipettes, meaning that they had a pre-established volume setting. After fixed volume pipettes, variable volume pipettes were introduced, which provided a more flexible stepper volume setting.

In 1972, Dr. Warren Gilson invented the first continuously adjustable variable volume pipette. As an original error-preventing feature, the selected volume was now clearly displayed on the Gilson pipette (direct digital readout). Today, Gilson precision pipettes are still the world standard for accuracy, precision, and reliability.

Gilson continues its creative efforts and offers innovative, durable, and reliable pipettes to help scientists in their daily work.

The pipetting system is our core expertise, and we truly enjoy sharing this knowledge and experience with you, and other pipette users, to help achieve your goals.

Filled with tips and information from our pipetting experts, this guide covers all aspects of pipetting, from pipette selection to proper maintenance. Examples given are based on our own pipette range, but the techniques described are equally applicable to other brands of pipettes and tips.

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Air-Displacement Pipette

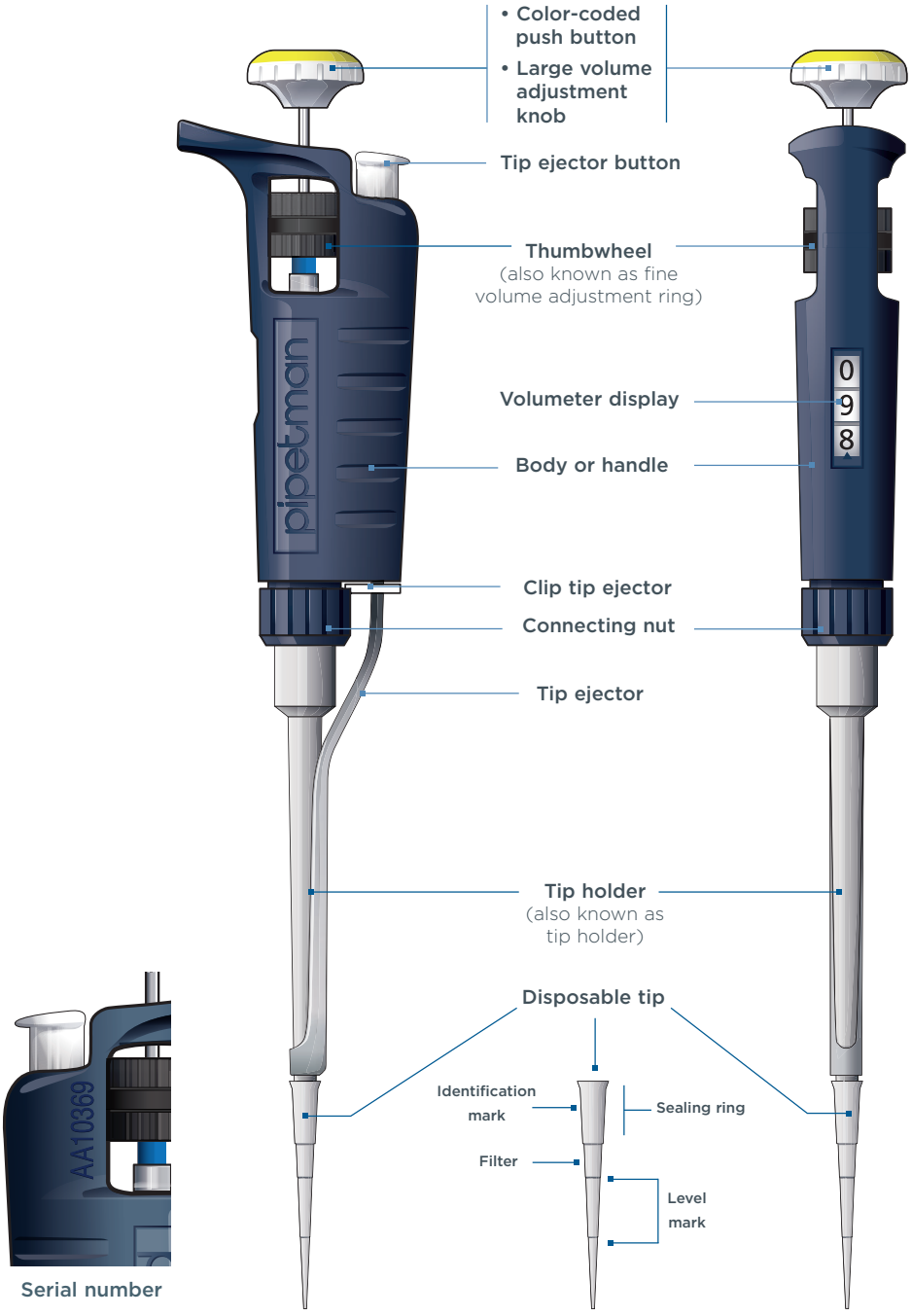


Figure 1
PIPETMAN® diagram*

*Pipettes with different designs are available. For more information, visit **www.gilson.com**.

Positive-Displacement Pipette

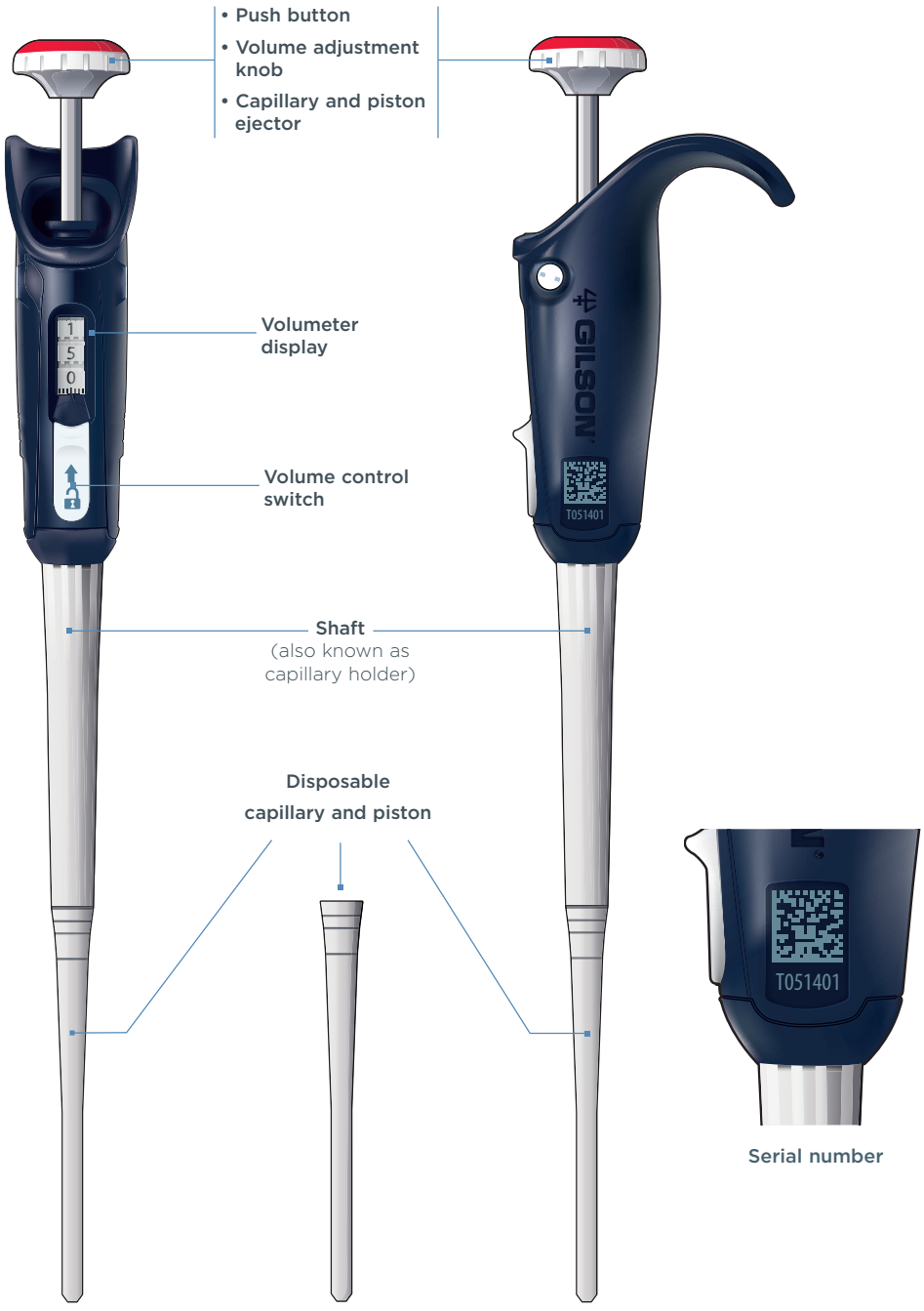


Figure 2
MICROMAN® E diagram*

SELECTING THE RIGHT PIPETTE

SELECTING THE RIGHT PIPETTE

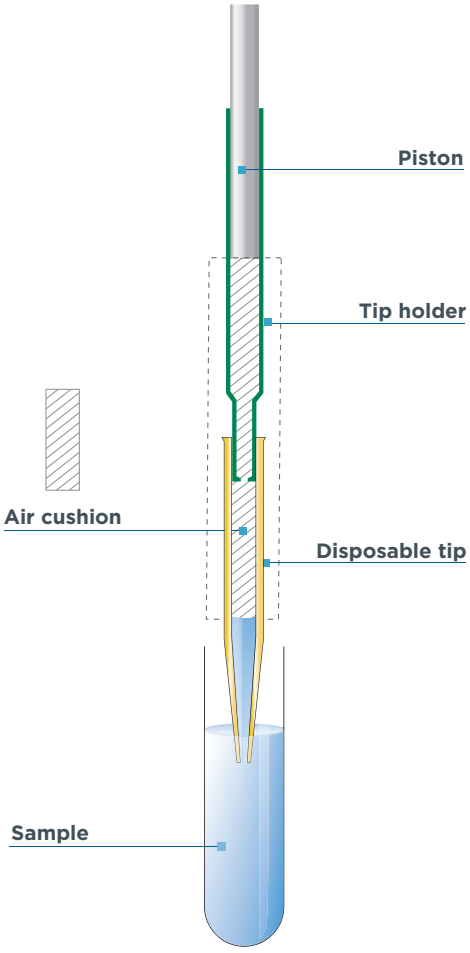
Working Principle of Pipettes

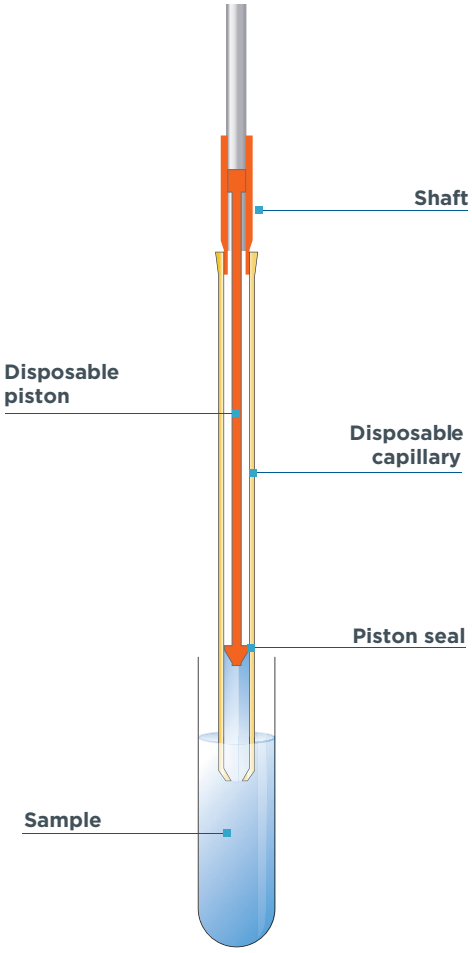
Air-Displacement Pipettes

- Recommended for aqueous samples and for general laboratory work.
- Cushion of air (dead volume) between the pipette piston and the liquid sample.
- The piston is integ rated into the lower part of the pipette.

Positive-Displacement Pipettes

- Recommended for non-aqueous samples (viscous, dense, volatile, radioactive, corrosive, contaminating, hot, and cold).
- Direct contact of the piston with the sample (no air cushion).
- The disposable piston is part of the tip (not integrated into the pipette).

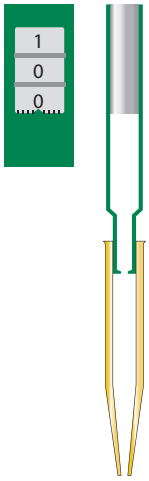




Working Principle of Air-Displacement Pipettes

When the push button is pressed on an air-displacement pipette, the piston inside the instrument moves down to let air out. This means the **air is displaced by the piston.** The volume of air displaced is equivalent to the volume of liquid aspirated.

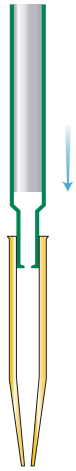
The following infographic shows how the piston determines the volume of air displaced and subsequently the volume of sample aspirated.



1

Set Volume

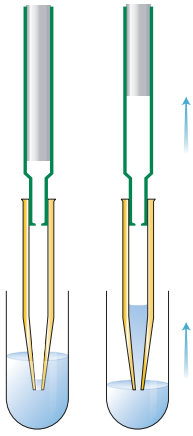
The required volume is set. The piston moves to the appropriate position.



2

Prepare for Aspiration

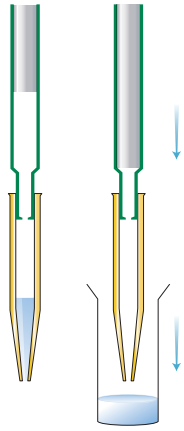
The push button is pressed prior to sample aspiration. The piston descends and expels a volume of air equal to the selected volume of liquid.



3

Aspirate Sample

As the push button is released, a partial vacuum is created inside the tip. The ambient atmospheric pressure forces the desired volume of liquid through the orifice into the tip.



4

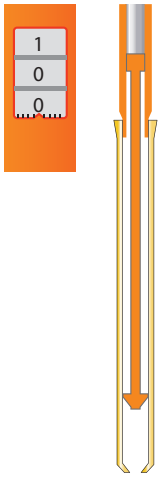
Dispense Sample

The push button is pressed again. Air pressure increases inside the tip holder and the tip.
The compressed air pushes the liquid out of the tip.

Working Principle of Positive-Displacement Pipettes

Positive-displacement technology, like the one used in our MICROMAN® E pipettes, is ideal when working with infectious, viscous, foaming, hot, cold, or volatile liquids because the disposable piston within the capillary piston (CP) tip is in direct contact with the sample.

The CP technology thus removes any air cushion inside the pipette and ensures the volume accuracy is not affected by the fluid's properties. This also helps prevent contamination and protects the sample, the user and the pipette.



1

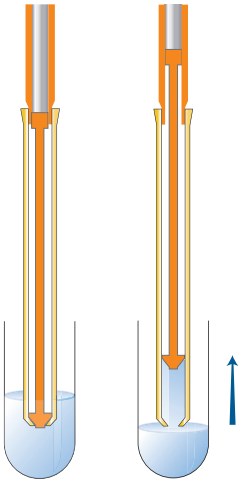
Set Volume

The required volume is set. The piston moves down to the appropriate start position.

2

Prepare for Aspiration

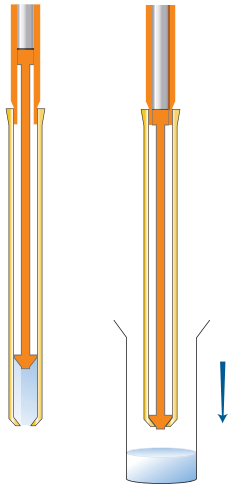
The push button is pressed prior to sample aspiration. The piston descends down to the end of the capillary.



3

Aspirate Sample

The orifice is immersed below the liquid surface. As the push button is released, the piston moves up and the ambient pressure forces the desired volume of liquid through the orifice into the capillary.



4

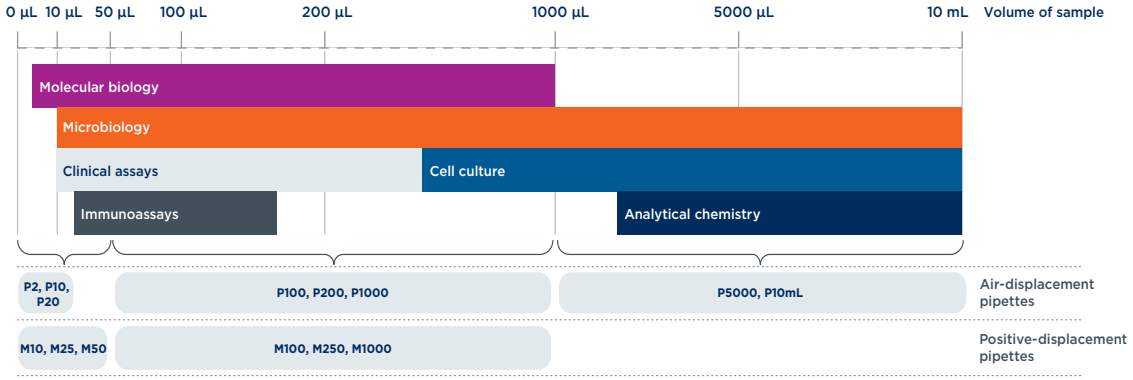
Dispense Sample

The push button is pressed again.The piston moves down and expels the liquid out of the capillary.

The Right Choice for Your Application

The type of analysis to perform, the physical properties of the liquid, and the volume to be handled will determine which pipette to use. It is recommended to select a pipette with a nominal (maximum) volume as close as possible to the desired volume to transfer.

Table 1
Recommendations for pipetting different volumes



Consider the Physical Properties of Your Sample

For volumes greater than 10 mL, it is suggested to work with a pipette filler like MACROMAN® with plastic or serological pipettes.

Regardless of the volume you require, the nature of the sample directly impacts precision and accuracy. Air-displacement pipettes are better for aqueous liquids whereas positive-displacement pipettes are used for non-aqueous samples such as viscous or volatile liquids.

SAMPLE TYPES	EXAMPLES	RECOMMENDED PIPETTES
Aqueous	Water, sucrose, Tris, buffers with a pH of 7	Air-displacement
Biological	DNA, RNA, proteins	Air-displacement with filter tips
Viscous	Glycerol, surfactants, oil	Positive-displacement
Volatile	Ethanol, hexane, formaldehyde	
Hazardous	Radioactive isotopes, blood, infectious bacteria or viruses	
Corrosive	Acids such as hydrochloric acid or sulfuric acid, bases such as ammonium hydroxide, salts such as sodium chloride	

Accuracy and Precision While Pipetting Non-Aqueous Liquids

Positive-displacement pipettes like MICROMAN® E are the right solution for complete and rapid pipetting of viscous and dense liquids such as oil, syrup, cosmetic cream, liquefied food, paint, glycerol, or buffers.

Positive-displacement pipettes are the unique solution to avoid leakage when pipetting high vapor pressure liquids such as acetone, chloroform, alcohol, or other solvents.

They also help prevent contamination and thus protect the sample, the user, and the pipette.

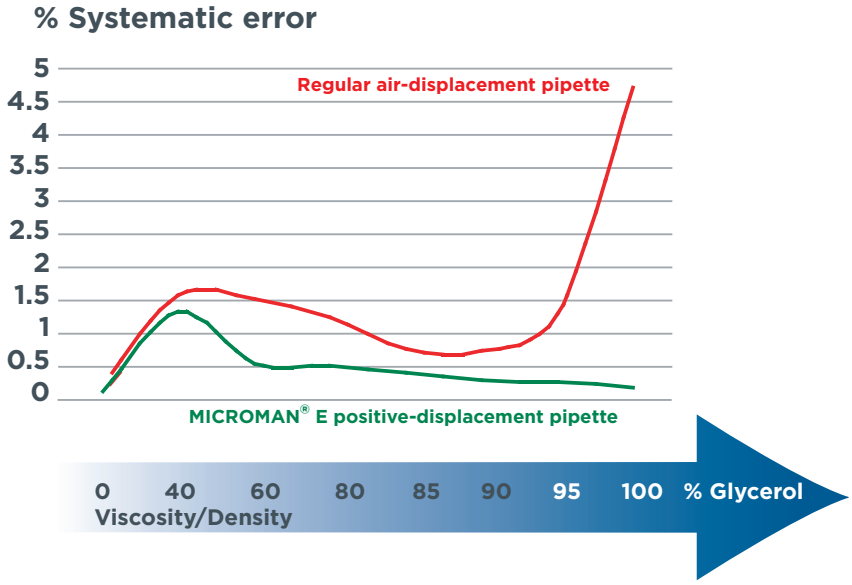


Figure 3
Systematic Error (%) when using a MICROMAN® E positive displacement pipette vs. a regular air-displacement pipette

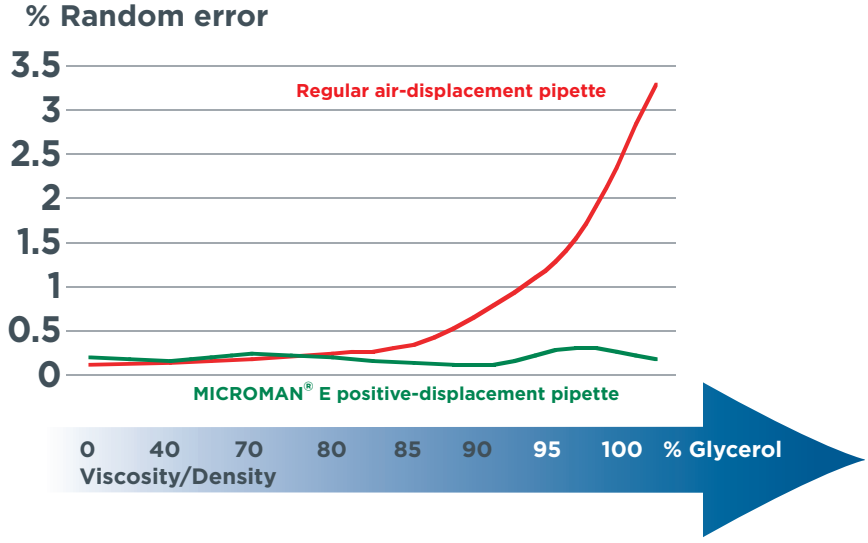


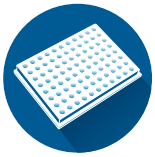
Figure 4
Random Error (%) when using a MICROMAN® E positive-displacement pipette vs. a regular air-displacement pipette

Specific Pipettes for Specific Vessels

Test tubes and centrifuge tubes are used with all single channel pipettes for sample preparations, such as qPCR workflow.

Long test tubes, are used with **positive-displacement pipettes and pipette fillers** with plastic or glass pipettes. These devices are specially designed to reach the bottom of the tubes.

Reagent reservoirs are ideal for dispensing reagents, especially with multichannel pipettes.



96- and 384-well microplates, as well as 8-well strips, are commonly used with **air-displacement, multichannel pipettes** for applications like ELISA.

They are also used with single channel pipettes.

Multichannel pipettes allow transferring 8 to 12 different samples at once and filling a microplate 8 to 12 times faster than with single channel pipettes.

High Throughput and Repetitive Pipetting

When pipetting in a high throughput setting, it is important to have reliable results and be as efficient as possible. Reliable results means not only having reproducible results with one technician's samples, but also among all technicians in the lab. There are a variety of ways to improve reliability and efficiency, some of which include using motorized pipettes and/or repetitive pipettes.

User-to-User Variability

Electronic pipettes can help reduce variability between users. There are many factors that can affect your pipetting results, including volume setting, pipetting technique, and the rate of aspirating and dispensing. With an electronic pipette you can set the exact volume on the digital display — the motor uses the same pipetting force every time and maintains the same rate of speed when aspirating and dispensing a sample.

Aliquoting

To deliver several aliquots without refilling, you may either choose the repetitive mode of **an electronic air-displacement pipette,** or **use a positive-displacement repeater.**

Repeaters enable up to 125 aliquots, whereas the number of aliquots with air-displacement motorized pipettes will depend on the pipette volume.

For operations fewer than ten aliquots, using a motorized air-displacement pipette is likely the best option.

PIPETTING TECHNIQUES

Adjust the Volume Display

Reading and Adjusting the Volume

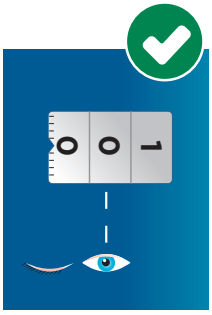
Hold the body of the micropipette in one hand and use the other hand to rotate the thumbwheel or the push button. With the push button, the volume can be easily adjusted with one hand. Push button volume adjustment is available on all MICROMAN E pipettes and on PIPETMAN pipettes (except PIPETMAN L) manufactured after April 1995.

A Helpful Hint for Improving Reproducibility and Accuracy

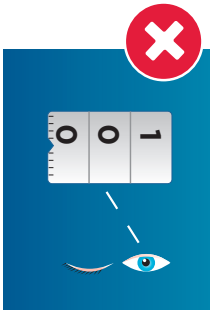
Always finish setting clockwise for best reproducibility. This is how to obtain a clockwise volume setting:

When decreasing the volume setting, slowly reach the required setting, making sure not to pass the setting.

When increasing the volume setting, pass the required value by 1/3 of a turn and then slowly decrease to reach the volume, making sure not to pass the setting.



Correct alignment: accurate reading



Incorrect alignment: error

To avoid parallax, hold the pipette in a horizontal position. Adjust the volume until the indicator is lined up with the desired volume.

NOTICE

To avoid internal damage to your pipette, never attempt to force the volume setting beyond the limits.

Air-Displacement / Forward Mode Pipetting

The forward mode is the standard way of pipetting with an air-displacement pipette like PIPETMAN.

1

Prepare

Hold the pipette in a nearly vertical position. Depress the plunger smoothly to the first stop position.

2

Aspirate

Immerse the pipette tip in the liquid.* Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.

3

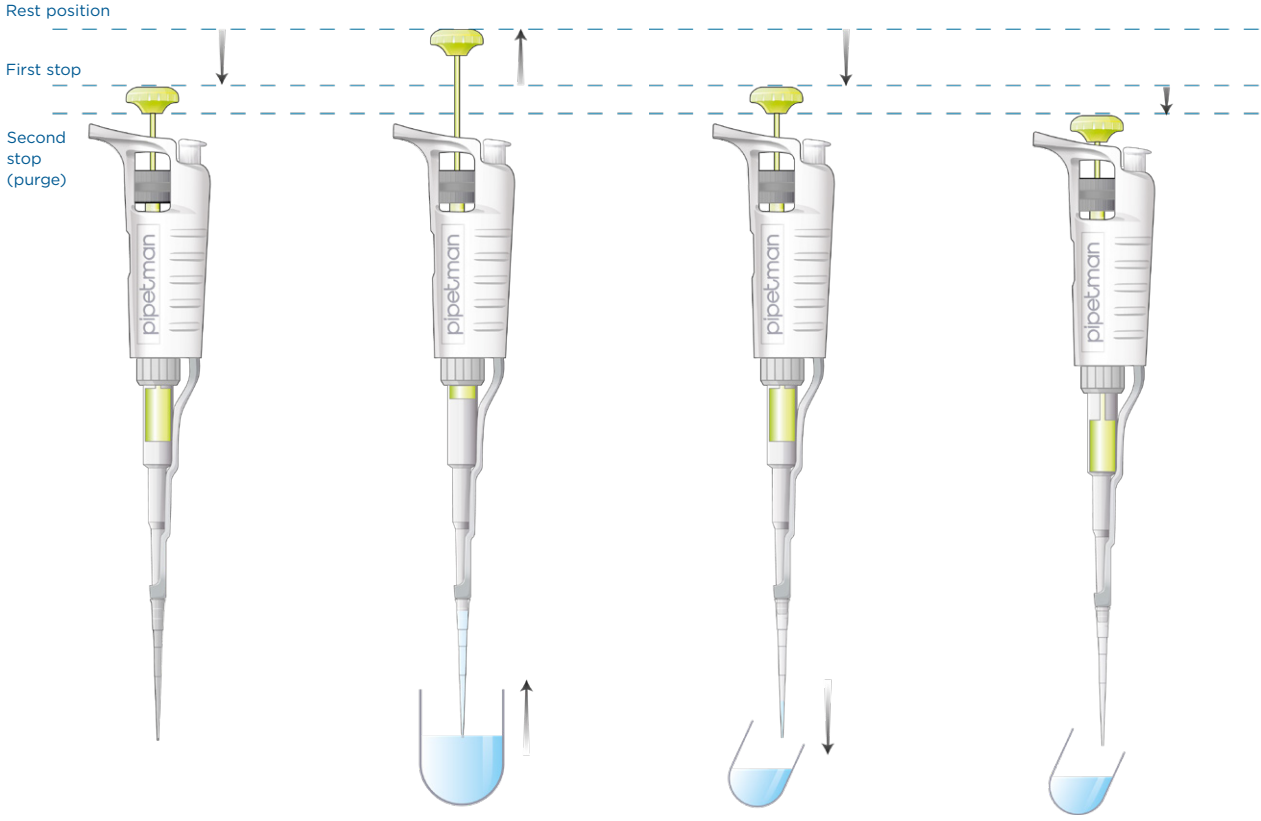
Dispense

Place the pipette tip at an angle (10° to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position.

4

Purge

Wait one second, then depress the plunger to the second stop position. This purge stroke removes any remaining sample from the tip. Remove pipette tip end from sidewall by sliding it up the wall.



* The immersion depth of your tip can have a significant effect on your results (for depth per model, see table above). If the tip is immersed too deeply, droplets will form on the outside of the tip and they will be deposited along with your sample. If the tip is not immersed deeply enough, vortexing will occur and your pipette will not aspirate the selected volume.

In general, precision in forward mode depends on precise draining by air pressure (air-displacement pipettes) or internal wiping of the pipette barrel (positive-displacement pipettes).

5

Home

Allow the plunger to move up to the rest position.

VOLUME	IMMERSION DEPTH
0.1 —1 µL	1 mm
1—100 µL	2–3 mm
101—1000 µL	2–4 mm
1001 µL— 10 mL	3–6 mm

Pre-Wet

Pre-wetting the tips before pipetting helps prepare the tips for the best pipetting performance. Ideally, the pre-wet includes both immersing the tip in the liquid and performing one pipetting step.

Pre-wetting the tips helps ensure that the volumes you pipette will be both accurate and precise within specifications.

Air-Displacement / Reverse Mode Pipetting

The reverse mode is only possible with air-displacement pipettes. It is sometimes used to pipette slightly viscous liquids.

1

Prepare

Hold the pipette in a nearly vertical position. Depress the plunger smoothly to the second stop position.

2

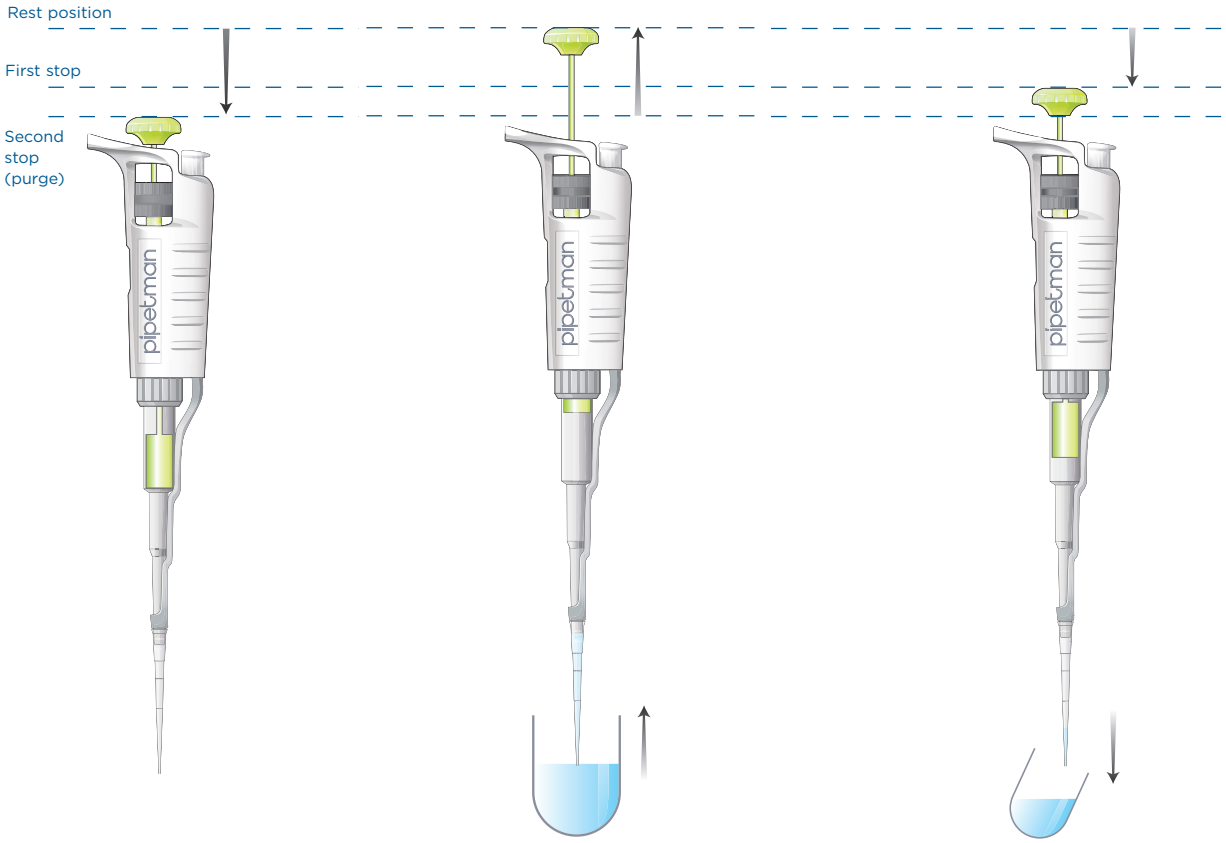
Aspirate

Immerse the pipette tip in the liquid. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.

3

Dispense

Place the pipette tip at an angle (10° to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position. Wait one second.



In reverse mode pipetting, the purge stroke is used during preparation. During aspiration, an amount of liquid equal to the amount of purged air is added. This amount compensates for the liquid that remains inside the tip while dispensing.

4

Re-Aspirate

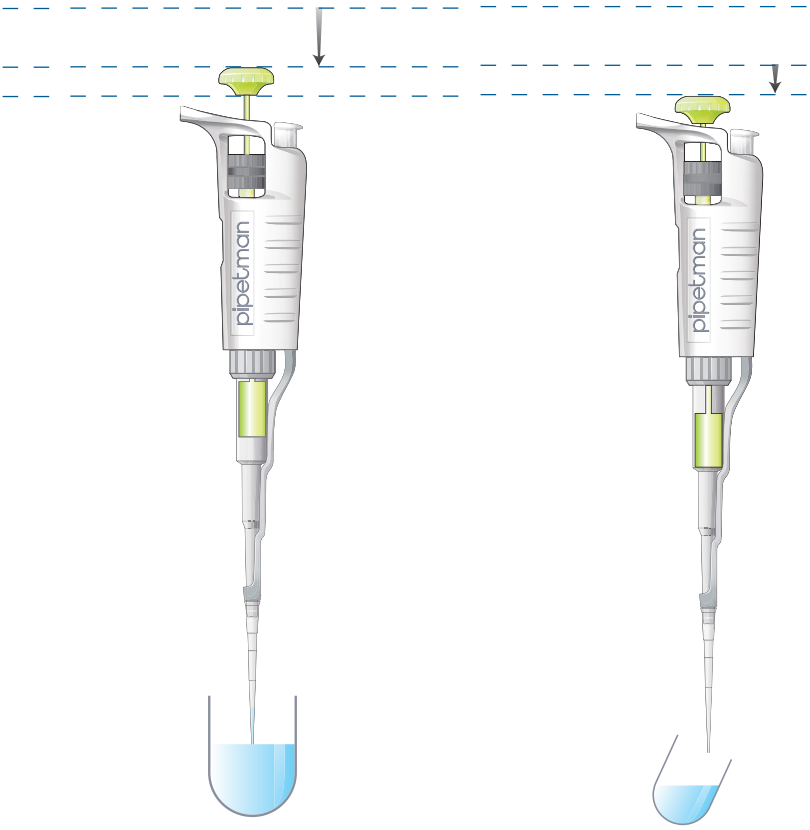
If the pipette tip is to be reused for the same sample, maintain the plunger in the intermediate position for subsequent immersion for the next pipetting cycle and restart step 2 (Aspirate).

or

5

Purge

Wait one second and then purge. If the pipette tip is not to be re-used, depress the plunger to purge position over an appropriate waste container and then eject the tip.



Positive-Displacement / Forward Mode Pipetting

The principle of the positive-displacement, and the absence of air cushion inside the pipette, enable a simple, quick, and reliable use of MICROMAN E.

Unlike air-displacement pipettes, MICROMAN E does not have any air cushion and there is no purge needed. Consequently, the Forward Pipetting mode is the only one adapted to the positive-displacement pipette (Reverse Pipetting mode is not suitable).

1

Prepare

Press the plunger button to the first stop. The piston moves to the appropriate position.

2

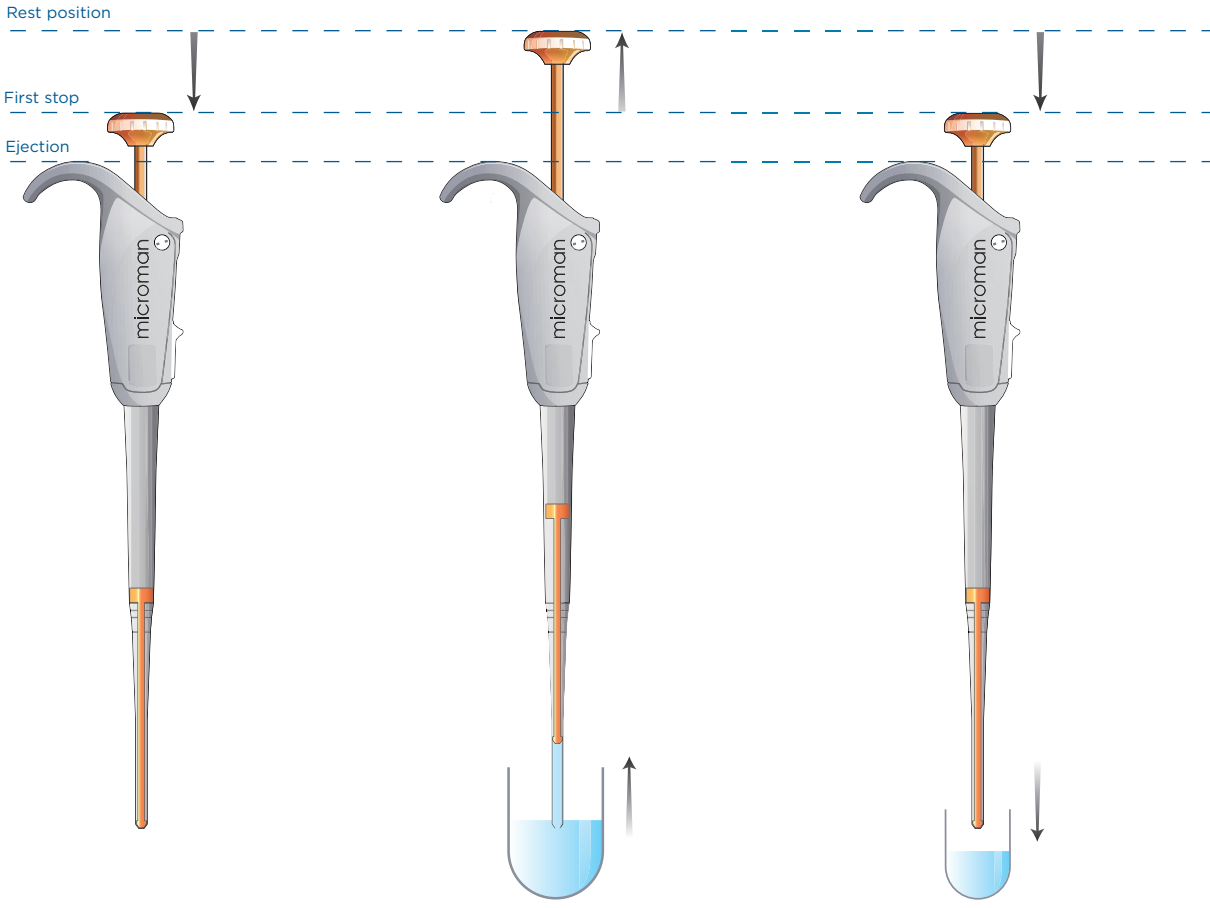
Aspirate

Immerse the capillary/piston in the liquid.* Release the plunger, letting it move up to the home position. The piston moves up and the ambient pressure forces the desired volume of liquid through the orifice into the capillary.

3

Dispense

Press the plunger button to the first stop. The piston moves down and expels the liquid out of the capillary.



* The immersion depth of your tip can have a significant effect on your results (for depth per model, see table page 20). If the tip is immersed too deeply, droplets will form on the outside of the tip and they will be deposited along with your sample. If the tip is not immersed deeply enough, vortexing will occur and your pipette will not aspirate the selected volume.

4

Eject

Press the plunger all the way down to the second and last stop. The capillary and piston are ejected without hand contact.

Wiping

To avoid distorted results linked to the volume pipetted, ensure that no liquid is on the outside part of the tip. When pipetting viscous liquids, such as cream, it may be necessary to wipe the outside of the tip or the capillary with a clean medical wipe. Do not touch the orifice. Choose a tissue that is resistant, lint-free, and inert to acids and solvents. Dispose of the tissue in a safe, hygienic manner.

NOTICE

When working with high risk specimens, do not wipe the disposable part. Make sure fluid depth penetration does not exceed the recommended immersion depth.*

Pre-Wet

To pre-wet, aspirate with the tip and then dispense back into the original reservoir or to waste.

NOTICE

When working with high risk specimens, do not wipe the disposable part. Make sure fluid depth penetration does not exceed the recommended immersion depth.

Tips for Mistake-Free Pipetting

How to Avoid Typical Pipetting Mistakes

MANY FACTORS MAY IMPACT PIPETTING ACCURACY

INFLUENCING PARAMETERS AND EFFECTS	CORRECTIVE MEASURES
Leaky/poorly seated pipette tips may affect accuracy by 0.5% to 50%	Use PIPETMAN® DIAMOND Tips or recommended pipette tips
Reuse of pipette tips may affect accuracy by up to 4%	Use pipette tips only once
The straightness of pipette tips may affect accuracy by up to 10%	Use appropriate tips only
The difference in vapor pressure of the liquid to be pipetted versus that of the water used for adjustment may affect accuracy by up to 2%	Pre-wet pipette tips
Failure to wipe pipette tip on the vessel wall can affect accuracy by up to 3%	Wipe of the pipette tip on the vessel wall (wiping distance 8 to 10 mm)*
Pipette tip immersion depth and handling angle during pipetting may affect accuracy by up to 1%	Hold pipette in a vertical position while pipetting
Irregular rhythm and timing during pipetting can affect accuracy by up to 1.5%	Apply a consistent pipetting technique
A leaky piston system can affect accuracy by 1% to 50%	Check the pipette and the volume aspirated regularly
Uneven piston movement can affect accuracy by up to 0.5%	Smooth pipetting of piston

Information extracted from ISO 8655-2 - Appendix B

* Gilson recommends touching the tip to the vessel wall at an angle of 10° to 45°.

Pipetting Ergonomics

Take a Few Minutes to Get Organized and Ensure You Have:

1. **An appropriate posture**
2. **The right equipment**
   Gilson offers various pipettes with forces adapted to user preferences. The forces of PIPETMAN L are some of the lowest.
3. **The appropriate technique**
4. **A good work organization and environment**

A good test for proper ergonomics is to see if you can rest your elbow comfortably on the work surface. If not, your receptacle may be too low or too high—find the right height.

Download the Gilson Ergonomic Poster on **www.gilson.com**

Take Time to Relax

1. If possible, try to switch periodically between different types of work.
2. Keep an appropriate, unrushed working speed. Let go of the pipette from time to time and give your fingers/hand a (micro) break.
3. Take frequent short breaks. Change your sitting position. Lean back and relax your shoulders and arms.

Ensure Smooth Pipetting

1. To facilitate uniform timing and motion, keep all necessary items within arm's reach.
2. Place **the most frequently used objects** in front of you. The more rarely used items can be placed a little further away from you.
3. The opening of the receptacle for used tips should be at the same height as the end of your pipette.

Use a Pipette Holder

Protect your pipette and always store it vertically on a pipette holder. Pipettes left on a workbench or stored in a drawer can easily come into contact with samples and become contaminated.

Figure 5
SINGLE™ pipette holder

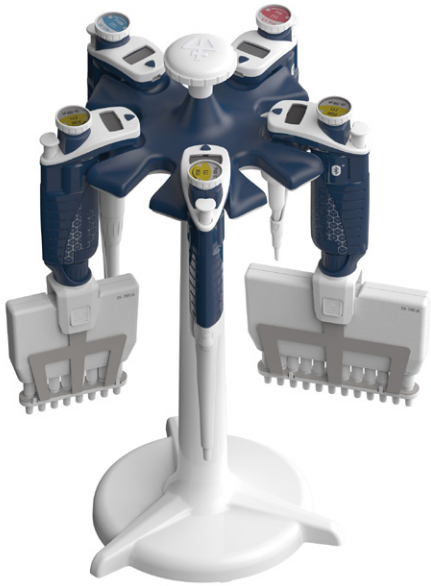


Figure 6
POWER CAROUSSEL™ stand

SELECTING THE RIGHT TIP

Fitting a Pipette Tip

Press down with a rotating motion

Avoid hammering the tip into the pipette

To Fit a Pipette Tip on a Single Channel Pipette

Hold the micropipette in one hand, lower the pipette into the tip, and use a slight twisting movement to seat the tip firmly on the tip holder of the micropipette to ensure an air-tight seal.

To protect your pipette, avoid tapping the tip onto the pipette like a hammer. Tips are available in TIPACK racks for easy mounting with no hand contact.

Exert a light vertical force followed by a slight lateral rocking movement to secure the tip fitting

To Fit a Pipette Tip on a Multichannel Pipette

To avoid damage to your pipette, Gilson does not recommend hammering or pounding on the tips.

The ROCKY RACK technique, invented by Gilson, available only in our TIPACK, TOWERPACK, BLISTER REFILL and RELOAD PACK makes it easy to fit tips on a multichannel pipette. Tips will not fall off nor will they have to be positioned manually.

Figure 7
Fit disposable pipette tips on single and multichannel pipettes

To Fit a Capillary Piston (CP) on a MICROMAN E

Pipetting with a MICROMAN E is as easy as using an air-displacement pipette. The CP can be fit onto the pipette in only two steps thanks to the patented QuickSnap system (which reduces and secures the number of steps required to fit a CP). The process is summarized in Figure 8:

1. Press the MICROMAN E into the CP until it is firmly secured and pick up the CP from the rack 1.
2. Slowly press the push button until there is a slight click and continue to press to the first stop 2.
3. Press the push button until the second stop 3 to eject the CP, and avoid any contact with the disposable CP.

For maximum protection against contamination, CPs for MICROMAN E pipettes are available in bulk no assembled, assembled rack, sterile, and non sterile.

Figure 8
CP fitting on MICROMAN® E

Ejecting the Used Tip

To avoid touching contaminated tips, hold the pipette over the waste container and press the tip ejector push button.

To eject the tip from MICROMAN E, depress the push button completely to the second stop. Discarded tips contain liquid residues, particularly when a pipette is used in reverse mode. Take suitable precautions when discarding disposables.

Figure 9
Ejecting the tips

When to Change a Tip

When transferring single samples of different liquids, select a new pipette tip for each new liquid.

It is strongly recommended to:

- Pre-wet every new pipette tip at the beginning of the test series to maintain reproducibility.
- Dispose the tip after each utilisation for accuracy and precision, as well as preventing cross contamination, when applying sensitive methods like PCR. For repetitive dispensing of the same liquid (diluent, buffer, or reagent), use the same pipette tip. This method is economical and efficient.

Gilson Pipette Tips

Gilson pipette tips are available in a variety of packaging options to suit virtually all needs and applications.

Refer to the Gilson tips selection guide to get a clear view of our entire pipette tips offering on www.gilson.com.

Autoclavable Pipette Tips

Bulk

- An economical solution for routine applications. May be hand loaded in empty tip racks for convenience or for autoclaving in the laboratory.

Racked for easy mounting with no hand contact

- TIPACKs have a hinged lid to protect against dust.
- Convenient 96-well format for filling microplates with multichannel pipettes and color-coded for easy identification.
- Ready for autoclaving in the laboratory.
- Tip racks may be reused.

Racked and sterilized for working in sterile conditions

- Factory sterilized and delivered in a sealed tip rack.

Racked refill

- TOWERPACK is a one hand system operation.
- High quality tips in an economic, easy-to-use, and eco-friendly rack refill system.
- The reload box is reusable and can be repeatedly autoclaved.
- Also available in sterilized packaging.

Sterile Pipette Tips

Sterile pipette tips are available in racked and individually wrapped versions.

They are available with filter and without filter.

Racked pipette filter tips are sterile and present the following advantages:

- Tips with a filter prevent contaminants such as aerosols from entering the pipette.
- Gilson filter tips are factory sterilized and delivered in a sealed rack.
- Sterile pipette tips are used for special application to avoid contamination.

STERILPACKs are individually wrapped and sterilized pipette tips.

- Opened just before use so the benefit of sterilization is assured right up to the last minute.
- A good solution when you only need a few tips.

Pipette Tips Material*		
Models	Tips	Filter
PIPETMAN DIAMOND Tips 0,1-1200 µL	Polypropylene (PP)	Polyethylene (PE)
AmpliPur Expert Tips 0,1-1200 µL	Polypropylene (PP)	Polyethylene (PE)
PIPETMAN Expert Tips	Polypropylene (PP)	Polyethylene (PE)

*For a complete list, please refer to the Gilson Consumable Data in www.gilson.com.

Capillary Pistons

- Combined with its disposable capillary piston tips (CP), the MICROMAN E positive displacement pipette works like a syringe. The CP eliminates the air cushion between the sample and the piston so volume accuracy is not affected by temperature or pressure changes or samples properties.
- Superior choice over air-displacement pipetting when using liquid types such as viscous (ex: oil, honey, cream, etc.), volatile, hot or cold, foaming, toxic (ex: blood, infectious bacteria, etc.), and corrosive (ex:hydrochloric acid).
- Capillary pistons protect pipette and samples against contamination.

Capillary Piston Material**		
Models	Capillary	Piston
1-10 µL	Polypropylene (PP)	Liquid Crystal Polymer (LCP)
3-25 µL	Polypropylene (PP)	Liquid Crystal Polymer (LCP)
20-50 µL	Polypropylene (PP)	Liquid Crystal Polymer (LCP)
10-100 µL	Polypropylene (PP)	Polyethylene (PE)
50-250 µL	Polypropylene (PP)	Polyethylene (PE)
100-1000 µL	Polypropylene (PP)	Polyoxymethylene (POM)

** The table is provided to verify chemical compatibility of the material. Refer to Appendix E: Chemical Resistance of Plastics on page 50 for more information. For a complete list, please refer to the Gilson Consumable Data in www.gilson.com.

Sterilization of Consumables by Beta, Gamma, or X-Ray Radiation

1. **Beta, gamma radiation of consumables**
   Sterilization of pipette tips is conducted according to ISO 11137-1:2006 requirements for development, validation, and routine control of a sterilization process for medical devices The sterilization can take place by exposure to gamma, beta, or X-ray radiation.
   The appropriate method is selected according to the material used to manufacture the product and does not leave any contaminant. Gilson consummables are pre-sterilized or sterilized by radiation. For more detail, please refer to the certificate provided with the product. Certificate of sterilization can be downloaded on www.gilson.com.

2. **Ethylene oxide gas (EtO)**
   If the type of plastic to be sterilized cannot withstand beta or gamma radiation, ethylene oxide (EtO) is used instead. EtO is notably used to sterilize CPs.

Gilson Pipette Tips Packaging

Gilson offers a wide range of tips packaging to suit all your needs.

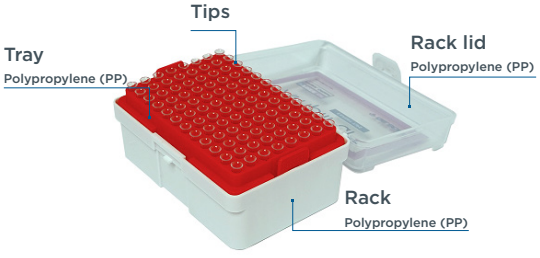


Figure 10
TIPACK description

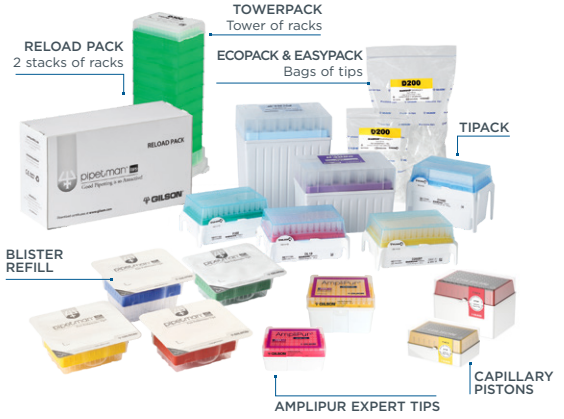


Figure 11
Gilson pipette tips packaging options

Evaluating Tip Quality

Although they may look alike, all tips are not equal. The choice of a poor quality tip may jeopardize your results. Choose the pipette tip recommended by the pipette manufacturer for the best accuracy, precision, and tip fit, and always check the following points:

Quality of the Tip's Raw Material

There are many different brands of tips made of different quality plastics. Gilson selects a specific polypropylene because it is naturally hydrophobic and a low retention material.

Absence of Potential Contaminants

Cleanness of tips is very important as production residues, such as dust or biological contaminants coming from the production site, may contaminate your samples. Additionally, tips should be chemically resistant and free of additives, such as silicone, dyes, biocides, antistatic agents, as well as traces of metal, such as aluminum, nickel, or zinc.

A trace metal certificate can be obtained from the manufacturer upon request.

Tip Manufacturer's Guarantees

GUARANTEEING TRACEABILITY

With the batch number on each box and bag, the history of the tips can be traced from packaging to delivery to the laboratory.

GUARANTEEING PRODUCTION QUALITY

Every Gilson pipette tips is individually marked with an identification number. With this number, the mold can be identified, and even the exact cavity that produced the tip can be located.

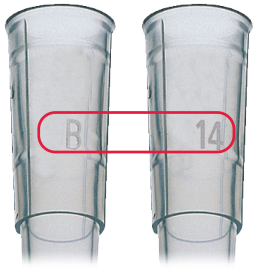


Figure 12
Gilson pipette tips guaranteed traceability

Figure 13
PIPETMAN® DIAMOND Tips and AmpliPur® Expert Tips identification label

PREVENTING CONTAMINATION

Types of Contamination and How to Prevent Them

Personal Protective Equipment

PREVENTION

The specific personal protective equipment recommended depends on your laboratory and can include:

- Lab coat
- Gloves
- Protective glasses
- Mask
- Protective footwear

LAB BENCH

- Wipe lab bench before and after your experiment with an appropriate cleaner for your application (cell culture, radioactive components, pathogenic samples, etc.)
- Work under a hood.
- Work behind a radioactivity shield.
- Avoid touching used tips.

Pipette to Sample

Contaminated tips or a contaminated pipette will contaminate your samples.

PREVENTION

- Store pipettes vertically on a holder.
- Eject tips into a designated container.
- Follow laboratory protocol to clean your pipette.
- Use sterile tips when appropriate.
- Change the tip after each sample to avoid cross contamination.

Sample to Pipette

Contamination can occur if the sample or aerosols from the sample are allowed to enter the body of the pipette.

PREVENTION

- To prevent your sample from contaminating the body of your pipette, do not turn the pipette upside down when there is sample in the tip. Always store your pipettes vertically.
- Release the push button slowly.
- Use filter tips to reduce contamination risk.
- Use the corrosion protection kit available for PIPETMAN P1000 (PIPETMAN Neo, PIPETMAN G, and PIPETMAN L).
- For complete protection of the pipette, choose MICROMAN E when working liquids that can produce aerosols.

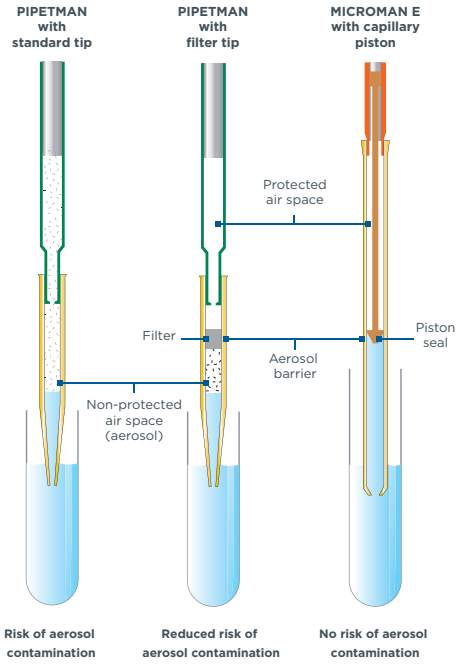


Figure 14
Solutions against contamination

Sample to Sample (Carryover)

CHANGE THE TIP AFTER EACH SAMPLE

A portion of sample "A" can adhere to the inside wall of the tip after sample delivery.

The leftover portion of sample "A" can mix with sample "B" and may cause a false test result.

Prevent Aerosol Contamination

It is essential to prevent aerosol contamination when using PCR and other amplification methods, or when pipetting DNA/RNA solutions, infectious materials, radioactive samples, etc.

Gilson offers two solutions:

1. **Use a PIPETMAN pipette with Gilson pipette filter tips when:**

- Working under sterile conditions.
- Pipetting aqueous samples.
- Avoiding cross-contamination.

2. **Use a MICROMAN E pipette with Gilson capillary pistons when:**

- Working under sterile conditions.
- Pipetting liquids that can produce aerosols.
- Avoiding cross-contamination.

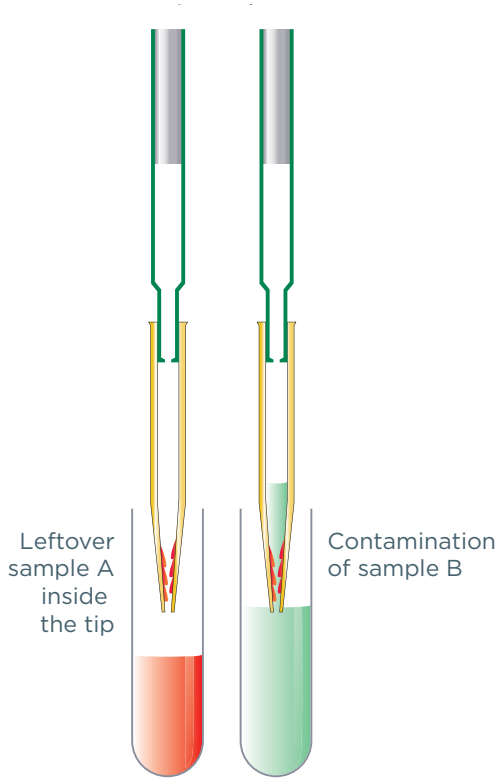


Figure 15
Sample carryover

Decontaminating Your Pipette

The solutions mentioned below are options—other solutions may be used. Make sure your decontamination technique is compatible with your pipette material and refer to your laboratory decontamination procedure.

CONTAMINATION CAUSES	DECONTAMINATION TECHNIQUES	CLEANING GUIDELINES
Radioactive compounds	Detergent or cleaning solution	Disassemble the lower part of your pipette. Fully immerse the contaminated parts* in an ultrasonic bath with a detergent or cleaning solution recommended for laboratory instruments. It is strongly recommended to rinse the pipette several times with water and dry it thoroughly. Always make sure that radioactivity has decreased to an acceptable level.
Viruses, bacteria, mycoplasma, fungi	UV radiation	Work surfaces may be decontaminated by exposure to 300 nm UV light for 15 minutes. UV will not damage Gilson PIPETMAN materials. Note that the UV rays cannot penetrate inside the pipette and cannot be considered as a decontamination protocol for the internal components of the pipette.
DNA, RNA, biological samples	10% bleach solution or UV radiation	Disassemble the lower part of your pipette. Fully immerse the contaminated parts* in at least 3% sodium hypochlorite for at least 15 minutes. Rinse thoroughly with distilled water and dry. Exposure to UV light for 30–60 minutes will further reduce DNA contamination, but not fully eliminate it from the pipette surface.
Aqueous solutions and buffers	Water cleaning	Disassemble the lower part of your pipette. Rinse the contaminated parts thoroughly with distilled water and dry.
Acids/alkalis		
Organic solvents	Detergent or cleaning solution	Disassemble the lower part of your pipette. Fully immerse the contaminated parts* in an ultrasonic bath with a detergent or cleaning solution recommended for laboratory instruments. Rinse the pipette several times with water and then dry it thoroughly.
Proteins		

If pipette brands other than Gilson are used, please make sure the material is compatible with the appropriate cleaning solutions.

* Check the User's Guide for specific parts to clean by immersion.

Autoclaving

This is a common method of sterilization. Gilson pipette non filter tips* and parts of PIPETMAN pipettes may be sterilized in the laboratory under the following conditions: moist heat/121°C/20 minutes/1 bar.**

NOTE **Autoclaving has a limited spectrum of action and will not destroy RNase, for example.**

*Sterile pipette tips, gel loading tips, plastic wrap, and tray of the TOWERPACK are not autoclavable.

** PIPETMAN parts can be autoclaved according to the User's Guide for the pipettes. Refer to your model User's Guide for the specific parts and the recommended conditions.

PIPETTE SERVICE AND MAINTENANCE

PIPETTE SERVICE AND MAINTENANCE

Pipette Specifications According to ISO 8655

The ISO 8655 standard gives the accuracy and precision limits as both absolute and relative values. Specifications will depend on the technique used (air-displacement, positive-displacement, or repetitive pipettes).

What are Published Manufacturer Specifications?

Specifications are established by the manufacturer. They guarantee, in terms of accuracy and precision, the performance of all pipettes of a given brand and a given model at a certain volume setting.

EXAMPLE OF MAXIMUM PERMISSIBLE ERROR FOR A 1000 µL PIPETTE		MAXIMUM PERMISSIBLE ERRORS GILSON		MAXIMUM PERMISSIBLE ERRORS ISO 8655	
MODEL	VOLUME (µL)	SYSTEMATIC ERROR (µL)	RANDOM ERROR (µL)	SYSTEMATIC ERROR (µL)	RANDOM ERROR (µL)
P1000	100 500 1000	± 3 ± 4 ± 8	≤ 0.6 ≤ 1 ≤ 1.5	± 8 ± 8 ± 8	≤ 3 ≤ 3 ≤ 3

These specifications are defined for pipettes used in forward mode. The gravimetric method is used with the temperature of the distilled water and all other conditions stabilized between 15°C and 30°C. The values given include all components of error due to both normal hand warming and the changing of the tip.

NOTICE **To comply with the ISO 8655 standard, the specifications of the pipette must be within the maximum permissible errors.**

Repair in the Lab or Return for Service?

PROBLEM	SOLUTION
Your pipette is more than one year old, and records show that it has not been serviced within the past 12 months.	The conformity to the acceptable maximum permissible errors should be tested at least once a year (ISO 8655-1). If you do not have the required equipment or if the pipette fails a performance check, return the pipette to your Local Gilson representative for service. Between service periods, Gilson recommends performing a Two-Minute Inspection (Refer to **Quick Pipette Diagnosis** on page 37).
For models other than microvolume pipettes (from 2 to 10 µL), you have identified damage to the push button, connecting nut, piston seal, O-ring, tip holder, or tip ejector.	Spare parts may be ordered from your local Gilson representative. These parts can be replaced on site without any impact on the performance of your pipette.

For all other damage, and for microvolume pipettes (2–10 µL), return the pipette for service.

Quick Pipette Diagnosis

Pipette Maintenance

Regular maintenance and service of your pipette ensures reliable results. Refer to your product User's Guide for manufacturer's recommendations.

TWO-MINUTE INSPECTION

Use the PIPETMAN Quick Inspection video and the Two-Minute Inspection poster to diagnose defects and decide whether the pipette should be repaired on site or returned to your representative for service. Direct any maintenance or service questions to your local Gilson distributor.

Refer to the Two-Minute Inspection video and the poster for a walk through of basic pipette performance evaluation.

Good routine maintenance helps prevent costly repairs.

Watch the Quick Inspection videos: www.youtube.com/user/GilsonPipetman

How to Calculate Volumetric Accuracy and Precision

"Accuracy" and "precision" are qualitative terms. The corresponding quantitative terms are "systematic error" and "random error." Conversion from weight to volume must be calculated first.

Evaluation of Accuracy

The specified accuracy is the limit to the systematic error, which is the difference between the mean volume of actual measurements and the true value of the volume set on the instrument.

The systematic error (E) can be estimated as follows:

$$E = \bar{V} - V_0$$

- E systematic error
- V_0 nominal volume
- $\bar{V}$ mean volume

$$\bar{V} = \frac{\sum_{i=1}^{n} V_i}{n}$$

- V_i individually measured volume
- n number of measurements

The accuracy of a pipette can be expressed as a percentage of the nominal volume:

$$E\% = \frac{\bar{V} - V_0}{V_0} \times 100$$

NOTE

Factors affecting accuracy:

Temperature
(temperature difference between delivery device and liquid)

Sample density
(affects the liquid volume aspirated into the tip)

Pipetting technique

Evaluation of Precision

The specified precision is the limit to the random error, which is the distribution of the measured values around a mean value. For pipettes, precision refers to a within-series group of data, and therefore to repeatability.

The random error is then quantified by the standard deviation of measurements performed at a given volume setting under the same measuring conditions. The standard deviation (SD or "s") can be estimated as follows:

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (V_i - \bar{V})^2}{n - 1}}$$

- $\bar{V}$ mean volume

The precision of a pipette can also be expressed as a percentage of the mean volume. This is known as relative standard deviation (RSD) or coefficient of variation (CV), and is estimated as follows:

$$CV = \frac{SD}{\bar{V}} \times 100$$

The mean value and number of replicates must be stated, and the experimental procedure used must be described in such a way that other workers can repeat it.

Pipette Calibration

Pipette calibration should be carried out by trained personnel on a regular basis to quantify your pipette performance.

Calibration of Pipettes in a Quality System

The main objective of pipette calibration in a quality system is to ensure that dispensing is carried out with the intended accuracy. Frequently, error limits are adopted from the manufacturer's specifications, although far less accuracy is needed to perform the task at hand. It should be kept in mind that in an uncontrolled laboratory environment, the manufacturer's specifications may not be achieved.

Consequently, users should define their own acceptance limits according to the application involved and ambient conditions. Another option is to use the acceptance limits stated in standards, such as ISO 8655. The actual standard specifications, and for highest accuracy the manufacturer's specifications, should be used only when testing can be performed in a controlled environment using distilled or deionized water.

Device Requirements and Test Conditions

An analytical balance must be used. The scale graduation value of the balance should be chosen according to the selected pipette volume. The ISO 8655 standard states the accuracy and precision limits as both absolute and relative values.

The values are specified for fixed single channel air-displacement pipettes. With variable volume pipettes, the nominal volume is the maximum selectable volume.

The microliter limit of the nominal volume applies to every selectable volume throughout the volume range. For example, for a 10–100 µL pipette the maximum permissible accuracy limit (systematic error) is 0.8 µL and the maximum permissible precision limit (random error) is 0.3 µL. With multichannel pipettes these values are doubled.

Procedure to Check Calibration

The pipette is checked with the nominal volume (maximum volume), approximately 50% of the nominal volume, and with the minimum volume specified by the manufacturer or 10% of the maximum volume, whichever is higher.

If the calculated results are within the selected limits, the adjustment of the pipette is correct.

Calibration with the Gravimetric Method

The gravimetric method is recommended by pipette manufacturers and international standard organizations (ISO 8655). It is based on the determination of the weight of water samples delivered by the pipette.

Implementation of this method requires the strict monitoring of environmental conditions and the systematic use of adequate and controlled equipment.

General Considerations

Gilson pipettes are designed to compensate for the effects of normal hand warming during the test series. However, the instrument being evaluated must not be over-warmed by extensive handling.

The temperature should be 21.5 ± 1.5°C (293–296 K)

Relative humidity should be maintained at 50%–75% in order to reduce the evaporation rate and to control the build-up of electrostatic charges

NOTE

If the pipettes are used and therefore checked outside these conditions, the weight of the setting volume of water aspirated will have to be corrected according to the conversion table (µL/mg). See appendix.

Recommended Equipment

- **Calibrated thermometer with a standard uncertainty of max 0.2°C**
  A calibrated thermometer readable to 0.1°C to measure both ambient and water temperatures at the beginning and at the end of the test series.
- **Hygrometer with a standard uncertainty of max 10%**
  A calibrated hygrometer to check the constant of humidity in the air during the test.
- **Barometer with a standard uncertainty of max 0.5 KPa**
  A calibrated barometer to check the atmospheric pressure.
- **Distilled water**
  Use distilled or deionized water conforming to grade 3 as specified in ISO 3696, degassed or air-equilibrated. The water shall be at room temperature.
- **Balances**
  Laboratory balances required for the test should meet or exceed the following performances:

SELECTED VOLUME (V) OF APPARATUS UNDER TEST	BALANCE RESOLUTION mg	REPEATABILITY AND LINEARITY mg	STANDARD UNCERTAINTY OF MEASUREMENT mg
1 µL < V ≤ 10 µL	0.001	0.002	0.002
10 µL < V ≤ 100 µL	0.01	0.02	0.02
100 µL < V ≤ 1000 µL	0.1	0.2	0.2
1 mL < V ≤ 10 mL	0.1	0.2	0.2

- **Vessels**
  Test equipment should correspond to the following indications:

EXAMPLES OF INSTRUMENTS	VOLUMES	SAMPLE RESERVOIR	WEIGHING VESSEL	BALANCE RESOLUTION	OTHER EQUIP.
P2 - P20 PM x20 F2 - F10 M10 to M25 M100	0.1 to 20 µL	Ø 35 mm H 50 mm	Ø 10.5 mm H 13 mm	0.001 mg	Lid Tweezers Filters
P100 - P200 F25 - F200 PM x300 M50 - M250	> 20 to 200 µL	Ø 35 mm H 50 mm	Ø 21 mm H 50 mm	0.01 mg	Lid
P1000 - P5000 F250 - F5000 M1000	> 200 to 5000 µL	Ø 50 mm H 70 mm	Ø 35 mm H 50 mm	0.1 mg	Lid
P10 mL	> 5 to 10 mL	250 mL beaker	Ø 40 mm H 100 mm	0.1 mg	Lid

Some Remarks About Balances

With modern analytical balances, a laboratory needs only two balances to check an entire stock of pipettes ranging from 0.1 µL to 10 mL. A good combination would be one six-digit balance and another one that works on two scales, for example 50 g with 0.01 mg sensitivity and 200 g with 0.1 mg sensitivity.

The test balances should be calibrated, maintained, and approved by your country's department of weights and measures.

To minimize vibration, the balances should be set up on a marble table. Keep the balance area free of drafts and the ambient area free of dust.

From Weight to Volume

Conversion to volume must consider the density of the liquid as well as evaporation during the cycle time. For each measurement, the corresponding volume (V_i) can be calculated as follows:

$$V_i = (W_i + \bar{e})\, Z$$

W_i is the weight as read on the balance.

$\bar{e}$ is the mean evaporating loss during the cycle time.

Z expressed in µL/mg, is a conversion factor incorporating density of water buoyed in air, at test temperature and barometric pressure.

For your reference, a complete example is given in APPENDIX A on page 45.

NOTE For measurements higher than 50 µL, the evaporation factor can be disregarded.

Estimation of the Z Factor (Conversion Factor)

The Z factor is not just equal to the density of water adjusted to the local temperature and pressure parameters. It must also consider the air density and the density of the weights used to calibrate the balance.

For very low volumes, application of the Z factor may not affect the final result.

The detailed formula, as well as the table indicating the Z factor to be considered, are provided in APPENDIX B on page 47.

Estimation of the E Factor (Evaporation Loss)

Evaporation that occurs during the gravimetric test depends mainly on temperature, humidity and cycle time of work. It may have a noticeable effect on small volume measurements (50 µL or less).

Evaporation loss is estimated by running a series of four simulated weighing cycles and calculating the mean weight loss per weighing cycle in milligrams. Perform each weighing cycle without adding the aspirated liquid to the vessel.

Dispense the liquid into a dummy vessel. The mean evaporation, $\bar{e}$, is calculated as follows:

$$\bar{e} = \frac{1}{4}\,(e_1 + e_2 + e_3 + e_4)$$

A procedure for the determination of $\bar{e}$ is given in APPENDIX C on page 48.

Performance Check Procedure

When to Perform the Test?

Since accuracy and precision have a direct influence on the quality of analytical results, it is imperative that the performance of individual pipettes be compared regularly with the manufacturer's specifications.

Gravimetric analysis is a practical, widely used method for testing the performance (accuracy and precision) of a pipette.

FREQUENCY*	CONTROL	ACTION	WHO
Daily	Preventive maintenance	Leak test (see the Two-Minute Inspection poster)	End-users
Weekly to up to every three months	Preventive maintenance	Accuracy check Cleaning & Diagnosis: - Visual inspection - Function check	End-users
Annually	Complete maintenance	Replacement spare parts of first level (seals/O-rings, tip holder...) Adjustment - Calibration	Gilson Authorized Service Center and End-users
		Replacement spare parts of second level (Piston, volumeter, operating rod) Adjustment - Calibration	Gilson Authorized Service Center

* Frequency should be adapted to the type of sample, the number of pipetting tasks, and the environmental conditions of the laboratory.

NOTE **Always check your pipette for mechanical faults (Refer to TWO-MINUTE INSPECTION on page 37) before performing a gravimetric test.**

1. Pour distilled or deionized water from the container into the weighing vessel to a depth of at least 3 mm.
2. Record the test conditions (ambient and water temperature, relative humidity, barometric pressure).
3. Select the test volume of your variable-volume, piston pipette.
4. Fit the tip or capillary/piston assembly to the pipette (the manufacturer's specifications are valid only when the test is performed with the manufacturer's tips).
5. Pre-wet pipette tip five times to reach humidity equilibrium in the dead volume of the pipette, but do not consider for calculations.
6. Change tip.
7. Pre-wet the tip once.
8. Pipette the test volume.
9. Determine tare mass (reset balance).
10. Open balance door, retrieve weighing container, deliver sample, replace on the balance and close the door.
11. After allowing display to stabilize, record the weight.
12. Repeat the test cycle until ten measurements have been recorded as a series of weights W_1 to W_{10}.
13. For samples below or equal to 50 µL, estimate evaporation loss by repeating steps 8 to 10 exactly as a normal sample weighing, but without actually adding any sample to the weighing container. Record absolute value (e_i) and repeat several (m) times.
14. Record test conditions. Check that values are still within recommended limits.
15. Use the average of the first and the second values of temperature and barometric pressure to determine the correction needed (Z).
Refer to **APPENDIX B** on page 47.
16. Calculate the accuracy and the precision and compare with manufacturer's or ISO 8655-2 specifications. (To calculate accuracy and precision, refer to **HOW TO CALCULATE VOLUMETRIC ACCURACY AND PRECISION** on page 38.)

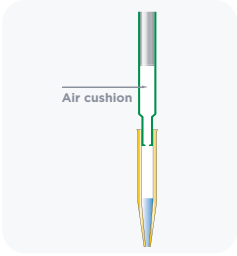
APPENDICES

Appendix A: Pipetting Terms

Accuracy*

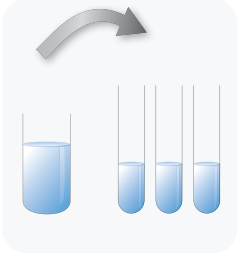
Closeness of agreement between a measured quantity value and a true quantity value of a measurand.
Note: "accuracy" is a qualitative concept.

Air Cushion



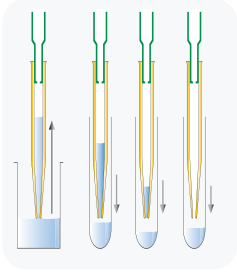
Also called "dead volume", the air cushion is the volume of air located between the lower part of the pipette piston and the surface level of the sample.

Aliquot



Measured portion of a homogeneous entity. A general term referring to multiple samples of any solution, mixture, etc.

Dispenser



An instrument for delivering predetermined volumes of liquid from a reservoir. The reservoir may be integrated into the instrument or connected externally.

Measurement Error*

Measured quantity value minus a reference quantity value.

Comment: this difference or deviation (positive or negative) may be expressed either in the units in which the quantity is measured (absolute error), or as a percentage of the true value (relative error).

Random Error*

Component of measurement error that in replicate measurements varies in an unpredictable manner.

Notes:
1. Random error is equal to error minus systematic error,
2. Because only a finite number of measurements can be made, it is possible to determine only an estimate of random error.

Systematic Error*

Component of measurement error that in replicate measurements remains constant or varies in a predictable manner.

Notes:
1. Systematic error is equal to error minus random error,
2. Like true value, systematic error and its causes cannot be completely known.

Comment: systematic error quantifies the error of accuracy of a pipette.

Good Laboratory Practice

Good Laboratory Practice (GLP) is concerned with the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded, and reported.

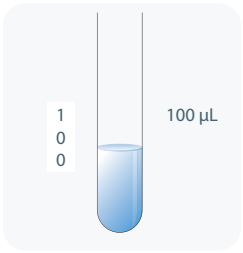
Measurand*

Particular quantity intended to be measured

Example: vapor pressure of a given sample of water at 20°C.

Note: the specification of a measurand may require statements about quantities such as time, temperature, and pressure.

Nominal Value*

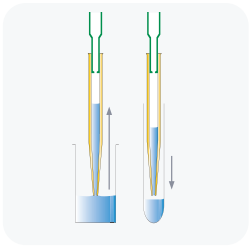


Rounded or approximate value of characterizing quantity of a measuring instrument or measuring system that provides guidance for its appropriate use.

Examples:

1. 1 L as the value marked on a single-mark volumetric flask
2. 100 µL as the setting appearing on the volumeter of a pipette

Pipette/Pipetter



An instrument for transferring a predetermined volume of liquid from one vessel to another. A pipetter is not connected to a reservoir.

Precision*

Closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions.

Repeatability* (of Results of Measurements)

Measurement precision under a set of repeatability conditions of measurement. Repeatability conditions include:

- Same measurement procedure
- Same operator/observer
- Same measuring instrument used under the same conditions
- Same location
- Repetition over a short period of time

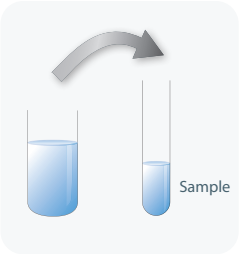
Comment: for pipetting, variations due to the operator (e.g., cycle time) are to be minimized.

*Definitions abstracted from VIM (International Vocabulary of Metrology).

Reproducibility* (of Results of Measurements)

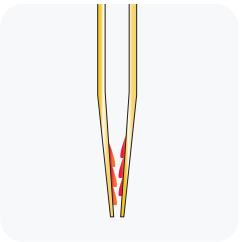
Measurement precision under reproducibility conditions of measurement.

Sample



The appropriate representative part of a liquid to be analyzed. The term "test sample" is used when necessary to avoid confusion with the statistical term “random sample from population”.

Sample Carryover



The portion of the sample that is retained in the instrument after sample delivery and that may affect subsequent samples.

Note: Carryover from a positivedisplacement pipette is less than from an air-displacement pipette.

Sterility Assurance Level SAL (ISO 11137)

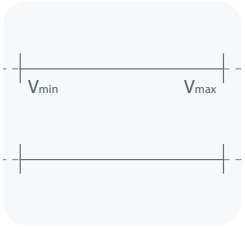
Quantitative value to assure sterility. SAL 10^{-6}: probability of 1 in 1,000,000 of finding a non-sterile unit.

- Sterile: refers to sterility assurance level SAL10^{-6}
- Pre-sterile: refers to sterility assurance level between SAL10^{-2} and SAL10^{-5}

True value

True value is a value that would be obtained by a perfect measurement.

Working range



Total volume and temperature range, as well as ambient conditions, for which instrument performance is specified.

Note: do not select volumes outside recommended limits.

Appendix B: Example of a Performance Check

Below is an example of how to evaluate the performance of PIPETMAN P10 at 1 µL.

1. Determine the mean value ē of the evaporation loss e_i that occurs during your pipetting cycles.
Proceed as described in Appendix D to determine e_i

$$\bar{e} = \frac{1}{m} \sum_{i=1}^{m} e_i$$

m : number of weighings

$e_1 = 0.016$ mg $e_3 = 0.021$ mg
$e_2 = 0.018$ mg $e_4 = 0.017$ mg

$\bar{e} = (e_1+e_2+e_3+e_4) / 4$

$\bar{e} = (0.016+0.018+0.021+0.017) / 4$

$\bar{e} =$ **0.018 mg/per cycle**

2. Change the pipette tip and perform the first weighing. Then, keep a regular cycle and perform the following ten measurements:

W_r = 0.957 mg

W_1 = 0.968 mg W_6 = 0.966 mg
W_2 = 0.960 mg W_7 = 0.955 mg
W_3 = 0.984 mg W_8 = 0.972 mg
W_4 = 0.942 mg W_9 = 0.958 mg
W_5 = 0.969 mg W_{10} = 0.967 mg

W_r rinsing measurement that is disregarded for the calculation

3. Calculate the mean weight

$$\bar{W} = \frac{1}{n} \sum_{i=1}^{n} W_i$$

n: number of weighings

$\bar{W}_i$ weighing results

$\bar{W}$ = (0.968 + 0.960 + 0.984 + 0.942 + 0.969 + 0.966 + 0.955 + 0.972 + 0.958 + 0.967) / 10

$\bar{W}$ **= 0.964 mg**

4. Calculate the mean volume
For a temperature of 21.5°C and an air pressure of 1013 hPa, the Z factor is equal to 1.0032 µL/mg (see table in Appendix C).

$$\bar{V} = (\bar{W} + \bar{e}) \times Z$$

5. Evaluate accuracy
Systematic error (**E**):

$$E = \bar{V} - V_0$$

V_0 true value on the instrument
E = 0.985 - 1= **0.015µL**

Relative error (E%):

$$E\% = (\bar{V} - V_0) \times 100 / V_0$$

E% = $(\bar{V}-V_0) \times 100 / V_0$
E% = (-0.015 x 100)/1 = **-1.50%**

6. Evaluate precision (repeatability)
Standard Deviation (SD_w)

$$SD_w = \sqrt{\sum_{i=1}^{n} \frac{(W_i - \bar{W})^2}{n - 1}}$$

$$SD_w^2 = \frac{1}{n-1} \sum_{i=1}^{n} (W_i - \bar{W})^2$$

$$SD_w^2 = \frac{1}{9} \left[\begin{array}{l} (0.968-0.964)^2 + (0.960-0.964)^2 + (0.984-0.964)^2 + \\ (0.942-0.964)^2 + (0.969-0.964)^2 + (0.966-0.964)^2 + \\ (0.955-0.964)^2 + (0.972-0.964)^2 + (0.958-0.964)^2 + \\ (0.967-0.964)^2 \end{array} \right]$$

Random error (**SDv**):

SD_w **= 0.011 mg**
$SD_v = SD_w \times Z$
SD_v = 0.011 x 1.0032 = **0.011 µL**

Appendix C: Z Factor

The reference calculation equation is: $Z = [1/(P_W - P_A)]\,[1-(P_A/P_B)]$

Where: P_A = density of air at t°C.
P_W = density of the test liquid at t°C.
P_B = density of the balance weights. Use 8 g/cc for PB

NOTE Weights conforming to International Recommendation No. 33 of OIML have been adjusted to give results when weighing in air as if the density of the weights were 8.0 g/mL.

Values of the conversion factor Z (µL/mg) as a function of temperature and pressure for distilled water.

Temperature °C	AIR PRESSURE HPA					
	800	853	907	960	1013	1067
15	1.0018	1.0018	1.0019	1.0019	1.0020	1.0020
15.5	1.0018	1.0019	1.0019	1.0020	1.0020	1.0021
16	1.0019	1.0020	1.0020	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0022	1.0022	1.0023
17	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024
18	1.0022	1.0023	1.0024	1.0024	1.0025	1.0025
18.5	1.0023	1.0024	1.0025	1.0025	1.0026	1.0026
19	1.0024	1.0025	1.0025	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0028	1.0028
20	1.0026	1.0027	1.0027	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0030	1.0030
21	1.0028	1.0029	1.0030	1.0030	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032
22	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0035
23	1.0033	1.0033	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0037
24	1.0035	1.0036	1.0036	1.0037	1.0038	1.0038
24.5	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039
25	1.0038	1.0038	1.0039	1.0039	1.0040	1.0041
25.5	1.0039	1.0040	1.0040	1.0041	1.0041	1.0042
26	1.0040	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0042	1.0043	1.0043	1.0044	1.0045
27	1.0043	1.0044	1.0044	1.0045	1.0045	1.0046
27.5	1.0044	1.0045	1.0046	1.0046	1.0047	1.0047
28	1.0046	1.0046	1.0047	1.0048	1.0048	1.0049
28.5	1.0047	1.0048	1.0048	1.0049	1.0050	1.0050
29	1.0049	1.0049	1.0050	1.0050	1.0051	1.0052
29.5	1.0050	1.0051	1.0051	1.0052	1.0052	1.0053
30	1.0052	1.0052	1.0053	1.0053	1.0054	1.0055

Appendix D: Evaporation Loss

Procedure for the Determination of Evaporation Loss.

Use the same distilled water, weighing vessel, and balance as you will be using for the gravimetric check.

1. Fill the weighing vessel half full with distilled water.
2. Cover the weighing vessel with its lid and place it on the balance using a pair of tweezers.
3. Aspirate a sample.
4. Tare the balance and take the weighing vessel out of the balance.
5. Use tweezers to remove the lid.
6. Dispense the sample into a dummy vessel.
7. Replace the lid on the weighing vessel and then use tweezers to place the the vessel on the balance.
8. Read the negative result e_1 (record the absolute value).
9. Repeat steps 3 to 8, three times to obtain e_2, e_3, and e_4.
10. Calculate the evaporation loss ee using the formula:

$$\bar{e} = \frac{1}{4} (e_1 + e_2 + e_3 + e_4)$$

Under normal conditions, this value is usually between 0.01 mg and 0.03 mg.

Appendix E: Chemical Resistance of Plastics

Product		Steel	PET	Nitril	EPDM	LCP	PA	PBT	PC	PE	PVDF	TPX	POM	PP
Acetamide		++	N/A	++	++	N/A	++	N/A	N/A	++	N/A	N/A	++	++
Ethyl acetate		++	+	-	++	++	++	++	++	++	++	+	N/A	++
Acetone		++	+	-	++	++	++	++	-	++	++	+	+	++
Acetonitrile		++	N/A	+	++	+	N/A	N/A	-	++	+	N/A	N/A	++
Acetic acid	20 %	++	++	+	++	++	++	N/A	++	++	++	++	++	++
	50 %	++	++	+	++	++	-	N/A	+	++	++	++	++	++
	100 %	++	++	-	++	+	-	N/A	-	++	++	+	+	++
Hydrochloric acid	10 %	-	++	++	++	++	-	++	++	++	++	++	++	++
	20 %	-	+	+	++	++	-	+	++	++	++	++	+	++
	37 %	-	-	-	++	++	-	-	+	++	++	++	-	++
Hydrofluoric acid	20 %	+	+	-	++	+	-	+	++	++	++	++	+	++
	40 %	-	+	-	++	-	-	-	+	++	++	++	+	++
Formic acid	100 %	++	N/A	-	++	++	-	+	-	++	++	N/A	+	++
Nitric acid	10 %	++	++	+	++	++	-	++	++	++	++	++	+	++
	30 %	++	+	-	+	++	-	+	++	++	++	++	-	+
	65 %	++	-	-	-	+	-	-	+	+	+	++	-	-
Phosphoric acid	20 %	++	N/A	+	++	N/A	-	++	++	++	++	++	+	++
	85 %	++	N/A	-	++	N/A	-	++	++	++	++	++	-	++
Propionic acid	50 %	++	-	+	N/A	N/A	++	++	+	++	++	N/A	-	++
	100 %	++	-	-	N/A	N/A	+	++	-	++	++	N/A	-	++
Sulfuric acid	20 %	++	++	+	+	++	+	++	++	++	++	++	+	++
	50 %	++	++	-	+	++	-	+	++	++	++	++	-	++
	95 %	++	+	-	-	-	-	-	+	+	+	++	-	+
Trifluoroacetic acid	20 %	++	N/A	-	N/A	N/A	+	N/A	++	++	++	N/A	++	++
	80 %	++	N/A	-	N/A	N/A	-	N/A	+	++	++	N/A	+	++
	100 %	++	N/A	-	N/A	N/A	-	N/A	-	++	++	N/A	-	++
Benzyl alcohol		++	++	-	N/A	N/A	+	N/A	-	++	++	++	-	++
Aniline		++	-	+	++	N/A	++	N/A	-	+	++	N/A	+	++
Butanol / Butyl alcohol		++	++	++	++	N/A	++	++	++	++	++	N/A	++	++
Chloroform		++	-	-	-	N/A	+	-	-	+	++	+	+	-
Cyclohexane		++	++	++	-	N/A	++	N/A	++	++	-	+	++	+
Diacetone alcohol		++	++	+	N/A	N/A	N/A	N/A	N/A	N/A	+	N/A	N/A	++
Methylene chloride		++	+	-	-	N/A	-	-	-	+	++	++	++	+
Diethylene glycol		++	N/A	++	++	++	N/A	N/A	N/A	++	++	++	++	++
Dimethylformamide (DMF)		++	++	-	+	++	++	++	-	++	-	++	++	++
Dimethylsulfoxide (DMSO)		++	N/A	-	N/A	N/A	+	N/A	-	++	N/A	N/A	N/A	N/A
Dioxane (1,4)		++	++	-	+	N/A	++	++	-	++	+	N/A	++	+
Ethanol		++	++	++	++	++	++	++	++	++	++	++	++	++
Ether		++	++	++	+	N/A	++	++	++	+	++	+	++	++
Formaldehyde		++	++	++	++	N/A	++	N/A	++	++	++	++	++	++
Hexane		++	N/A	++	-	+	++	++	++	+	++	+	++	++
Hydrogen peroxide	50 %	++	N/A	+	++	N/A	++	++	++	++	++	++	++	++
Ammonium hydroxide	20 %	++	++	++	++	N/A	N/A	+	-	++	N/A	++	++	++
Sodium hydroxide	10 %	++	+	++	++	++	++	+	-	++	++	++	++	++
	40 %	++	-	+	++	++	++	+	-	++	++	++	++	++
Sodium hypochlorite	15 % Cl	+	N/A	+	++	++	++	++	++	++	++	++	-	+
Methanol		++	++	++	++	+	++	++	+	++	++	++	++	++
Methyl ethyl ketone		++	++	-	+	++	++	++	-	++	-	+	+	++
Pentane		++	N/A	++	-	N/A	N/A	N/A	++	++	++	+	++	N/A
Tetrahydrofuran (TH-)		++	++	+	+	+	++	+	-	-	+	+	N/A	+
Urea		++	++	N/A	N/A	-	++	++	N/A	++	++	N/A	++	++

PET = Polyethylene Terephthalate
Nitril = Nitrile
EPDM = Ethylene Propylene
LCP = Liquid Cristal Polymer
PA = Polyamide
PBT = Polybutylene Terephthalate
PC = Polycarbonate
PE = Polyethylene
PVDF = Polyvinylidene fluoride
TPX = Polymethylpentene
POM = Polyoxymethylene
PP = Polypropylene
++ No chemical degradation
+ Medium resistance to chemical agents
- Low resistance to chemical agents
N/A No data available

FAQs

I have to pipette 100 µL and several pipettes can be used—which one should I choose?

According to the properties of the liquid to be handled, air-displacement or positive-displacement should be chosen.

- P100 is recommended for best precision.
- P200 can be used, but make sure that specifications are adapted to your protocol.

When should I pre-wet the tip?

Pre-wetting should be performed:

- Every time you change a tip
- Every time you increase the volume setting

How can I prevent piston corrosion?

- After contact with corrosive liquids, a piston should be cleaned with alcohol and a soft tissue. Take care to avoid shocks or scratches.

Use:

- Filter tips
- Corrosion protection kit
- Positive-displacement pipette

The tips I have do not fit well. What should I do?

- Always use Gilson brand tips for Gilson pipettes.
- Push upward on the tip ejector to make sure it is positioned properly.
- Clean the tip holder with alcohol. If it is worn or has been chemically attacked, order a new part from your local Gilson representative.

What is accuracy?

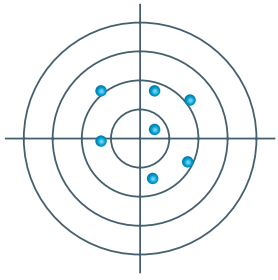
- Closeness of agreement between a measured quantity value and a true quantity value of a measurand.

Notes: "accuracy" is a qualitative concept.
Definition abstracted from VIM (International Vocabulary of Metrology).

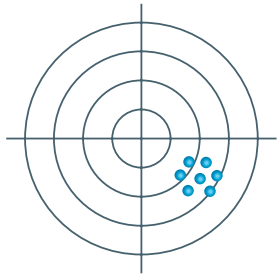
What is precision?

Closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions.

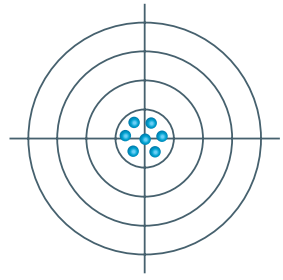
Note: Definition abstracted from VIM (International Vocabulary of Metrology).



Accurate but not precise



Precise but not accurate



Accurate and precise

NOTES

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